氏　　名	陸　彦因
授与した学位	博　士
専攻分野の名称	歯　学
学位授与番号	博甲第６６２０号
学位授与の日付	令和４年３月２５日
学位授与の要件	医歯薬学総合研究科機能再生・再建科学専攻 （学位規則第４条第１項該当）
学位論文の題目	Exosome-Based Molecular Transfer Activity of Macrophage-Like Cells Involves Viability of Oral Carcinoma Cells: Size Exclusion Chromatography and Concentration Filter Method (マクロファージ様細胞のエクソソーム分子送達能は口腔癌細胞の活性に関与する：サイズ排除クロマトグラフィー濃縮フィルター法の考案)
論文審査委員	久保田　聡　教授　　浅海　淳一　教授　　伊原木　總一郎　准教授

学位論文内容の要旨

Extracellular vesicles (EV) heterogeneity is a crucial issue in biology and medicine. In addition, tumor-associated macrophages are key components in cancer microenvironment and immunology. However, macrophage-derived EVs heterogeneity and their effects on receiver cancer cells have not been well understood.

In the present study, our aims were as follows: (i) to separate different sized EVs (such as small exosomes, large exosomes, and large EVs) from macrophage-like cells, (ii) to investigate the EV-based molecular transfer of palmGFP (approximately 30kD) to receiver oral carcinoma cells, and (iii) to examine whether the macrophage-EVs altered the cell viability of receiver oral carcinoma cells.

A human monocytic leukemia cell line THP-1 was differentiated to CD14-positive macrophage-like cells by stimulation with PMA (phorbol 12-myristate 13-acetate) but not M1 or M2 types.

We developed a combination method of size exclusion chromatography and concentration filters (SEC-CF) and aimed to characterize different EV types by their size, cargo types, and functions. Using the SEC-CF method, we first fractionated the culture supernatant into 20 fractions (Fractions 1, 2, 3…20). To simplify the EV analysis, we concentrated these fractions into the following three groups using concentration filters: Fr. 1–6, Fr. 7–9, and Fr. 10–20. In Fr. 1–6 of the particle size was between 100–500 nm and peaked at 208.9 nm, suggesting that Fr. 1–6 contained large EVs (larger than exosomes). In Fr. 7–9, the particle size ranged between 50–300 nm with a peaked size of ap-proximately 150 nm, suggesting large exosomes (EXO-L) from the size. In Fr. 10–20, the size of particles was smaller than 100 nm and peaked at approximately 40 nm, which could contain small exosomes (EXO-S).

To characterize the small and large exosomes or larger EVs using protein markers, we next performed Western blotting of tetraspanins (CD9 and CD63 are established EV markers), HSP90α, HSP90ß (often found in EVs), and ß-actin. CD9 was markedly detected in the Fr. 7–9. On the other hand, CD63, another tetraspanin family member often found in EV appeared in Fr. 10–20 (EXO-S).

We also established intercellular communication experiments using a conditioned medium (CM) and a transwell-based co-culture system. To confirm whether macrophage-secreted factors alter the recipient cell's viability, we examined the ATP content of the HSC-3 cells after receiving macrophage-secreted factors in the CM or in the co-culture system, and found macrophage-secreted factors decreased the viability of oral carcinoma cells. In addition, we prepared macrophage-derived EVs by SEC-CF method and confirmed that the macrophage- secreted EXO-S and EXO-L significantly reduced the cell viability (ATP content) in oral carcinoma cells.

Taken together, using SEC-CF method, we could separate different subtypes of EVs from macrophages: (i) rare large EVs (500–3000 nm) reminiscent of apoptosomes, (ii) EVs (100–500 nm) reminiscent of microvesicles (or microparticles), (iii) EVs (80–300 nm) containing CD9-positive large exosomes (EXO-L), (iv) EVs (20–200 nm) containing unidentified vesicles/particles, and (v) EVs (10–70 nm) containing CD63/HSP90-positive small exosomes (EXO-S) and particles. The SEC-CF method is useful for the purification of large and small exosomes with higher molecular transfer activities, potentially enabling efficient molecular delivery to target cells. In addition, the molecular transfer activities of exosomes from macrophage-like cells can reduce viability in oral carcinoma cells.

論文審査結果の要旨

Background: Extracellular vesicles (EV) heterogeneity is a crucial issue in biology and medicine. In addition, tumor-associated macrophages are key components in cancer microenvironment and immunity.

Objective: Since macrophage-derived EVs heterogeneity and their effects on receiver cancer cells have not been well understood, the aims of the thesis were as follows: (i) to separate different sized EVs (such as small exosomes, large exosomes, and large EVs) from macrophage-like cells, (ii) to investigate the EV-based molecular transfer of a palmitoylation signal-fused GFP (palmGFP) (approximately 30kD) to receiver oral carcinoma cells, and (iii) to examine whether the macrophage-EVs altered the cell viability of receiver oral carcinoma cells.

Materials and Methods: The applicant and collaborators developed a combination method of size exclusion chromatography and concentration filters (SEC-CF) and aimed to characterize different EV types by their size, cargo types, and functions. A human monocytic leukemia cell line THP-1 was differentiated to CD14-positive macrophage-like cells by stimulation with phorbol 12-myristate 13-acetate (PMA) but was not to M1 or M2 types. For a molecular transfer assay, they developed a THP-1-based stable cell line producing palmGFP associated with the membrane. The THP1/palmGFP cells were differentiated into macrophages producing palmGFP-contained EVs.

Results: Using the SEC-CF method, the following five EV types were fractionated from the culture supernatant of macrophage-like cells: (i) rare large EVs (500–3000 nm) reminiscent of apoptosomes, (ii) EVs (100–500 nm) reminiscent of microvesicles (or microparticles), (iii) EVs (80–300 nm) containing CD9-positive large exosomes (EXO-L), (iv) EVs (20–200 nm) containing unidentified vesicles/particles, and (v) EVs (10–70 nm) containing CD63/HSP90-positive small exosomes (EXO-S) and particles. For a molecular transfer assay, they developed a THP-1-based stable cell line producing palmGFP associated with the membrane. The THP1/palmGFP cells were differentiated into macrophages producing palmGFP-contained EVs. The macrophage/palmGFP-secreted EXO-S and EXO-L efficiently transferred the palmGFP to receiver human oral carcinoma cells (HSC-3/palmTomato), as compared to other EV types. In addition, the macrophage-secreted EXO-S and EXO-L significantly reduced the cell viability represented by ATP content in oral carcinoma cells.

Conclusion: Taken together, this thesis showed a SEC-SF method useful for the purification of large and small exosomes with higher molecular transfer activities, enabling efficient molecular delivery to target cells, which could be applied to targeted drug delivery system in future.

This article is already published in Cells and its scientific value is internationally recognized. Therefore, the thesis defense committee hereby accept this article as a doctoral dissertation in dentistry.